November 13, 1952

Dear Bruce:

Your letter of the 13th just received.

I do appreciate the typewriting, and hope you can continue to cultivate this excellent habit. It doesn't seem seriously to cramp your style. I made some notes in reply, point by point:

I have been asking Edwards about 541, 545, 546. If you leave space in the ms. I will insert the details when (and if) I get them.

d-f-g. Probably the paper should be written on the basis that FA-phage. Without overemphasizing it, it would do no harm to include some parentheses on the correlation with Boyd's phages. Norton has done a serum-neutralization; again, it would do no harm to mention it, but the full exposition should be reserved for his detailed account. Much the same should apply to the inclusion of phase-variation data. The agreement of H-reversions with the implied phase of the O-form should be emphasized, but no more than this,

I do think that tracks and flares should be mentioned to the extent of a photograph and a hypothesis. I am more convinced than ever that flares may come from selection for increased motility following the major gene transduction. Is there any comparison of spontaneous vs. transinduced swarms that would bear on this? Some motile old or rough motile strains show very nice flaring when first passed on motility agar.

I agree on the criteria for unmasking an O. I had in mind that using FA from a rare flagellar type would decrease the likelihood of getting the same type on the first trial, which would necessitate further study, with a second FA.

Thanks for Boyd's report. I've just recently receivedhis standard strains. I have found some R-specific phages, but these do not seem to transduce.

iv: Don't take this too seriously. The experiments have to be done much more carefully. The 666-FA response is drastically non-linear, probably connected with lysis. Re serum, Delbruck once found that the depressor effect could be reversed by serum after adsorption.

v: b-reactivity is still very puzzling. I have only a single broth culture that shows it well. It is a mixture of smooth and several kinds of rough (some colonially smooth, but rough re phage and transduction), The latter agglutinate non-specifically, and I have not been able to reconstitute the b-reacting mixture. I shouldn't be surprised if it turns out to be a fluke, our "b" reagent having some other nonspecific component.

vi: Would you also send Felix 546 as well as 534-588? The fermentation reactions (rhamn; inos) agree with 546--534, not #3 --534 as given in the stock book. From Norton's comments, the new identification does no violence to the records. If the cultures agree in Vi-type, it will help too, so I am sending SW-703 too.

viii: The difficulties in serial transduction largely predate the understanding of FA- phage. The only serious difficulty is in making FA from a strain. The ones that won't be transinduced were probably rough.

ix: Whatever you like. If necessary, I will criticize specific applications later.

xi: I am afraid many of my remarks have been confused by my not yet having cleared up the situation of b:i ratios in different crosses. The situation now seems fairly clear. FA (typhimurium) --X SW666 gives, as you know, mostly b (53 b: 2i in my last experiments). This is comparable also with your paratyphi A effect. On the other hand, the filial i's --X 666 give mostly i (8b: 43i). This swing in ratio is what made me think initially that only i was produced, contradicting the linkage hypothesis. There are at least two or three hypotheses: the filial FA involves an A^i H^+ segment that has been selected once for its linkage; in addition, it is necessarily adapted to paratyphi B (as the original PLT22 is not) and thus induces lysogenicity, though not invariably. I am further studying the correlation of transduction and induction of lysogenicity. PLT22 hardly ever does the latter, for SW-666. If the host-adaptation to SW-666 persists through a passage on LT-2, it may be possible to determine whether the linkage ratio reversal is related to the character of the phage or of the donor genotype. On the other hand, the two-step i transinduction (666 x- PLT22)b x-- PLT22 should serve to give an i that has not been selected for linkage. The only data I happen to have already are on (666 x- PLT22)b x- (666 x-PLT22)i i --x 666. (Following Whitehead and Russell, the orders of transduction can be indicated by x-, x--, x--- etc., in these complex formulae). These add up to 15 i : 3b, which contradicts nothing. What first looked like environmental effects on the ratios were confounded by the various FA's used for different experiments.

Anyhow, I now realize that I have not yet collected a significant body of data on the tymur^1 and tymur^2 --x 666 (Only 18/18 b from the latter, compared to 53/55 as mentioned above for the former). Perhaps, I have also been confused by the separate problem of the expression of A_2^{12} in monophasic genotypes. Even if correct, the recovery of i from $(3L46)^2$ --x 543 does not vitiate my hypothesis, as the "suppressed" A_1^{i*} from A_1^{i*} A_2^{12} may be capable of functioning, or rapidly losing the * in the 543 genotype. (I am also speculating on whether the determination of i or b in tymur -x 666 may not be based on a * sort of variational change rather than crossing-over, but have not perfected this hypothesis, waiting for the results of the previous paragraph.) The distinction between the FA of the two phases seems still to be holding up in further (limited) transduction experiments between diphasics (now including also abony -x sandai to give IX XII a:enx).

Norton also had some previous data bearing on this, in experiments -x typhi: FA tymur^2 -x typhi gave no swarms at all. Some of these were monophasic^2 (SW-547) = Edwards 191; others were serum-selected from diphasics. Still, the data are not so extensive as to generalize yet. I will not be unhappy if my working hypothesis turns out to be incorrect: it still gives me a tangible basis to bite into the problem. On the other hand, it has not yet been contradicted in the diphasics x-.

Did I claim to get enx into 543? Probably, I was referring to sandiego -x 543. The phase is probably eh (is being checked now) but my only reagent is enx, with which it reacts.

SW-546 is behaving for all the world like a A_1^{12} allele, but how it got that way I can't imagine. 546-x typhi gives a IX XII --1,2. Perhaps the most interesting (if correct) is one that is behaving like enx: 1,2 from abony^2 -x 546. Don't depend on this one.

Among other things, I am sending SW-905, 906, 908 resp. Hirsch's motile, paralysed and nonflagellated paratyphi B. The last is probably an independent mutant from 905, as it is readily transduced to motility (as well as rare sp. reversions). Cross-trans-motilizations are being tested, but go ahead yourself. The stocks are remarkable for the very high rate of phase variation: every colony of 905 or 906 reacts with both b and 1,2 ; one usually more than another in agreement with the inoculum. How would you go about measuring its rate?

--Continued, 11/21/52

Just received Edwards' report on the transductions, verifying most of what I've told you. The sand-diego --x SW666 is eh--. The "weak b" and other odd phases that I've mentioned are all z_{33}, the "third phase" of minnesota, and, according to Edwards a frequent j phase from b-carrying serotypes. I don't see any point bothering J Taylor about this, but will send one. A dublin -x typhimurium that I had recorded as gp turns out to be diphasic: gp:1,2. This fits the general hypothesis of separate loci for specific and nonspecific phases, but I am rather worried that this type of combination, now seen to be genetically possible, is not found in the diagnostic scheme. I shall have a start an epidemic with it.

Some of the experiments menioned above are done. PLT-22 adapted to 543 retains this adaptation when grown back on typhimurium. I'll call this 22B for the present, as it appears to be a host range mutant. 22B/LT2 behaves like 22/LT2 in -x 543, that is mostly b. Unfortunately, the (666 x- PLT22/2)b x-- PLT22/2 is resistant (lysogenic) to 22B, so that I will have to make another from which an FA can be made. SW666 lysogenic for 22B retains its susceptibility to transduction, as previously surmised.*

This is turning out to be an excellent system for correlating lysogenicity with transduction. I am in the midst of an experiment, but the first results are clear enough: 22B/609 -x 666 on EMB galactose. Of the Gal+ papillae, so far 13/20 are lysogenic; of nearby Gal- from the same plates, 5/29 are lysogenic. I expect that much of the lysogenicity may ensue from infection by second cycle phage, but the result is clear enough anyhow, and pretty tangible evidence correlating transduction with lysogenicity-inducing phage. Swarms are not such good material for this, as reinfection is even more likely to occur (judicious use of antiserum will help), but the general picture seems pretty much the same. An experiment on tracks is in the incubator. I must mention that unstable lysogenic types have recurred frequently. These produce mostly very rough-looking colonies, with an occasional smooth, nonlysogenic "segregant". I strongly suspect that early cases of very highly mutable R-S variation (E.g. Deskowitz) may have had a similar basis. I have not gotten another Galv transduction in 666, and the first one was not unstable for lysogenicity (Lp as I remember).

What was your indicator system for Boyd A1-A3. My first preps. had no action on 666 (lytic or otherwise), and are so far doubtful on SW-435: of course, my titres may be inadequate. We were certainly lucky with PLT22 to have such a wide range of action.

Edwards states that his #157 was isolated from a java strain about 10 years ago by intensive serum selection. I am trying to get the original.

If I remember correctly, PLT-7 appears in LT-7 following aging or penicillin treatment. Norton will probably tell you of his serum-inactivation experiment. I think I agree with him that the further characterization of FA as phage is a distinct subject, though I see no reason why it cannot be mentioned if immediately relevant to any point in the presently-considered paper,

*and promises to be an excellent strain for quantitative correlation of Gal+ and H+ transductions.

(For terminology, are you planning to use H_1 ; H_2 ; ... for the O-H factors, and A_1 A_2 for the flagellar antigens, or would H be better for this? We should settle this fairly soon.)

I don't ~~must~~ know what we are likely to do about Europe. I have accepted an invitation to prepare a longer review paper for the Genetics Congress, but don't know whether I will be able to read it in person.

The following SW- are being sent: FA made with PLT22 or 22B.

SW-803 abony Edwards 103

666 SW603 Gal-

662 553 -x 603 IV V XII gp:-

664 san diego -x 603 eh:-

668 san diego -x typhi H901 IX XII eh:-

676 673 selected with b serum. z_{33} (673 is equiv. 609, recurrence)

679 IV V XII gm enteritidis ex 666

674 553 -x 435 IV V XII gp:1,2

698 abony^{2} -x tymur LT-2 IV V XII i:enx

699 abony^{1} -x tymur LT-2 b:1,2

683 heidelberg -x 666 r

902 altendorf -x typhi H901 IX XII e:-

905 W. Hirsch 2859 S. paratyphi B b:1,2

906 1515 " paralysed b:1,2

908 2859-0 O-form

925 abony^{2} -x sendai IX XII a:enx

926 abony^{2} -x SW546 IV V XII enx:1,2 ! (The same type appears in the reciprocal cross).

(typhimurium^{1} -x 546 and abony^{1} -x 546 give ~~mix~~ i:- and b:- respectively). These results are mutually consistent in assigning A_1^{12} to SW-546. I do not know what allele is present at A_2, if any. Conceivably A_2^{12}, but this should give us i:1,2 from tymur-x546.)

May I have from you miscellaneous O forms (especially para A), and SL-46? In testing for linkage, it would be advisable to use FA from (typhimurium -x 543)i , as this favors the proportion of linked transductions.

Sincerely,

Joshua Lederberg